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Flooding-induced N_2O emission bursts controlled by pH and nitrate in agricultural soils

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Abstract

Agricultural soils are a major source of the greenhouse gas nitrous oxide (N_2O) to the atmosphere. Increasing frequency and severity of flooding as predicted for large intensively cropped areas may promote temporary denitrification and N_2O production but the effect of flooding events on N_2O emissions is poorly studied for agricultural systems. The overall N_2O dynamics during flooding of an agricultural soil and the effect of pH and NO_3^- concentration has been investigated based on a combination of the use of microsensors, stable isotope techniques, KCl extractions and modelling. This study shows that non-steady state peak N_2O emission events during flooding might potentially be at least in the order of reported annual mean N_2O emissions, which typically do not include flood induced N_2O emissions, and that more than one-third of the produced N_2O in the soil is not emitted but consumed within the soil. The magnitude of the emissions are, not surprisingly, positively correlated with the soil NO_3^- concentration but also negatively correlated with liming (neutral pH). The redox potential of the soil is found to influence N_2O accumulation as the production and consumption of N_2O occurs in narrow redox windows where the redox range levels are negatively correlated with the pH. This study highlights the potential importance of N_2O bursts associated with flooding and infers that annual N_2O emission estimates for tilled agricultural soils that are temporarily flooded will be underestimated. Furthermore, this study shows that subsurface N_2O reduction is a key process limiting N_2O emission and that a reduction in N_2O emissions is achievable if highly fertilized N-rich soils are limed.

1. Introduction

Future climate change will lead to changes in precipitation worldwide. A higher frequency of extreme rainfall events is predicted for temperate areas such as New Zealand and Northern Europe (IPCC, 2007; Min et al., 2011). This increases the risk of flooding for low-lying or poorly drained areas, which are the same areas receiving run off and ground water with potentially high nitrate (NO_3^-) concentrations. As a consequence it can be expected that there will be an increase in nitrous oxide (N_2O) production and emissions from these areas, particularly fertilized and nitrogen-rich agricultural fields (Knowles, 1982).

Nitrous oxide is a greenhouse gas with a global warming potential relative to CO_2 of 298 on a 100 year time horizon assuming a lifetime of 114 years in the atmosphere (IPCC, 2007). Additionally, N_2O has a negative effect on stratospheric ozone as NO and other free radical species (NO_x), generated from N_2O, deplete the ozone layer (Badr & Probert, 1993). The atmospheric concentration of N_2O has increased since pre-industrial times by 16% from 270 ppb to 319 ppb in 2005 (IPCC, 2007) and it is currently considered the dominant anthropogenic ozone depleting substance emitted (Ravishanakara et al., 2009). Soils are the main source of both anthropogenically and naturally produced N_2O and changes in land use have been the primary driver for the observed increase in tropospheric N_2O concentration (IPCC, 2007). Today, agricultural fields account for 42% of the total anthropogenic contribution of N_2O to the atmosphere and N_2O is the single most important greenhouse gas when looking at agricultural soils (IPCC, 2007).

In oxygen (O_2) limited environments production of N_2O in soil occurs as microbial processes utilize nitrogenous compounds as electron acceptors (Knowles, 1982). During denitrification N_2O is an obligate intermediary product in the reduction of NO_3^- to N_2, a

process performed by heterotrophic microorganisms. It is also a by-product during dissimilatory NO_3^- reduction (DNRA) to ammonium (NH_4^+) as NO_3^- is reduced to NH_4^+ via nitrite (NO_2^-) by fermentative microorganisms (Tiedje et al., 1982). Denitrification rates increase with organic C and NO_3^- availability, soil water content, pH and temperature (Knowles, 1982; Šimek and Cooper, 2002). The N_2O:N_2 ratio, describing the end product of denitrification, shifts in favour of N_2O as soil NO_3^- concentrations and acidity increase (Knowles, 1982; Weier et al., 1993). Not all N_2O produced in a soil will be emitted as it can be consumed during denitrification to N_2 a process controlled by the presence of N_2O reductase (NOS) (Knowles, 1982). Highly anoxic conditions, caused by high soil water content and high availability of easily degradable organic matter, favour the consumption of N_2O (Wrage et al., 2001) as NOS is strongly inhibited by the presence of O_2 (Knowles, 1982). Thus the balance between N_2O consumption and production rates controls N_2O emissions as well as the transport properties of N_2O in the soil (Clough et al., 2005). The primary mode of transport for N_2O in the soil is diffusion, which is controlled by concentration gradients according to Fick's law of diffusion.

The environmental factors for production of N_2O are optimal when fertilized fields are flooded. Non-steady state draining experiments have established the relationship between water-filled pore space and N_2O emissions (Castellano et al., 2010), however, to the authors knowledge, no studies on agricultural soils and only a few studies on natural soils have examined the effect of soil flooding on N_2O dynamics: the temporal and spatial trends of subsurface N_2O concentrations and net surface emissions. Jørgensen and Elberling (2012) found a distinct pulse pattern in N_2O concentrations and emissions when flooding an un-managed wetland peat soil. An increase in N_2O concentrations was observed within the first 24 hrs followed by a rapid decline in concentration, until the N_2O concentration was below

89 detection after 40 hrs. It was concluded that for these wetland peat soils the increase in N_2O
90 production would not affect the annual N_2O emission budget, even if flooding event
91 frequency increases in the future (Jørgensen and Elberling, 2012). This may not be the case
92 for agricultural fields, where tillage events can increase the availability of NO_3^--N (Eriksen
93 and Jensen, 2001; Silgram and Shepherd, 1999) and thereby the potential for N_2O production
94 via denitrification.

95 The aim of this study was to investigate the overall N_2O dynamics during a flooding event
96 of a New Zealand agricultural soil as affected by soil pH and NO_3^- concentration. Specific
97 aims of the study were to determine the balance between produced, consumed and emitted
98 N_2O from the soil and to determine the depth- and time-specific production and consumption
99 of N_2O. Two methods were used in combination to achieve the aims: depth-specific profiling
100 of the soil N_2O concentration and the redox potential using microsensors as well as 2 M KCl
101 extractions of 3 soil layers per soil core after ^{15}N labelled NO_3^- addition. The study was
102 designed based on the hypothesis that it is possible to mitigate N_2O emissions by changes in
103 agricultural practises (with a focus on changes in soil pH and N-input) and that annual N_2O
104 inventories made to date have potentially been underestimated because the impact of flooding
105 has not been included in annual budgets.

2. Materials and methods

A Templeton silt loam soil (Udic Ustochrept) was collected from a field, with a management history of perennial pasture, from the top layer (0-10 cm deep) during cultivation for pasture renovation, Lincoln, Canterbury (43^{o} 38.720S; 172^{o} 26.753E Lat/Lon). The Canterbury region is temperate with mean annual precipitation of 600-700 mm and a daily mean air temperature range of 1-10°C in the coldest months and 12-22°C in the warmest (Cappelen and Jensen, 2001). The Templeton soil and similar inceptisols represents app. 25% of the Canterbury Plains (Molloy, 1988). Inceptisols in temperate areas are soils with high inputs of fertilizer N (Potter et al., 2010) with crop types typically consisting of cereals such as barley and wheat (Leff et al., 2004).

The sampled soil was air-dried, sieved (< 2 mm) and kept dry and cold (4°C). The soil pH was determined (10 g air-dried soil:25 mL water). Half of the soil was treated with 2.08 g $Ca(OH)_2$ kg^{-1} dry soil (quicklime) in powder form to increase the pH by one unit. Lime treatment and the resulting pH increase were made consistently with previous experiments (Clough et al., 2003). Inorganic-N and dissolved organic carbon (DOC) were determined for both the un-treated and the limed soil. Inorganic-N was determined in a 2 M KCl extraction (4 g soil:70 mL KCl, shaken on an end-over-end shaker for 60 min and filtered through Whatman 42 filter paper. Filtered samples were analysed using an Alpkem FS3000 twin channel flow injection analyser (FIA) with Alpkem Winflow 4.03 software). The DOC was analysed by a DI water extraction (1:10 soil:water ratio), shaken on an end-over-end shaker for 30 min followed by centrifuging at 3500 rev/min for 20 min and filtered through a Whatman 42 filter paper into a 30 mL sample vial (Ghani et al., 2003). The DOC was determined based on the difference between the total organic carbon (TOC) and the inorganic

carbon (IC) analysed using a Shimadzu Total Organic Carbon Analyser (TOC-5000A) fitted with a Shimadzu ASI-5000A autosampler.

2.1 Core preparations

Soil was packed into either stainless steel metal cores (D = 7.4 cm) for microsensor measurements or PVC plastic cores (D = 7.5 cm) for KCl extractions (see below). Soil core bases were covered with a 1 mm nylon mesh and packed to a depth of 3.5 cm. The soil was packed in layers to ensure an even bulk density of 1 g cm^{-3} throughout the profile. Four treatments were made: control (soil with no additions, **TC**), limed soil (soil plus lime, **TL**), soil with N added (soil plus nitrate-^{15}N, **TN**), and soil with N and lime added (soil plus nitrate-^{15}N and lime, **TLN**). For treatment TN and TLN a known volume of ^{15}N enriched (50 atom%) KNO_3 solution (0.0154 M) was sprayed onto a designated mass of dry soil prior to packing the soil cores, supplying 100 µg NO_3-N g^{-1} soil. Since NO_3^- is evenly distributed in the surface of a cultivated soil, the ^{15}N-NO_3^- was applied to the entire depth of soil in the packed soil core. Soil cores were packed and adjusted with KNO_3 immediately before flooding. The soil cores were then flooded from below immediately prior to commencement of microsensor measurements, to mimic the rise of a high groundwater table, by placing them in a water-filled box. This method of soil flooding also minimised soil drainage during wetting. In total 108 cores were made, of which 12 were used for microsensor measurements and 96 for KCl extractions. KCl extractions were performed on three replicates at eight time steps for each treatment. The timing of the KCl extraction was distributed throughout the pulse of N_2O production (see supporting information (SI) (Fig. S1)). Due to measurement constraints of the microsensor, replication was done in time by sequentially measuring one soil core from one treatment at any given time. For each treatment a total of 3 replicates were measured at t_1, t_2 and t_3. In practice, two replicates of each treatment (at t_1 and t_2) were

153 measured after each other. All t_1 and t_2 measurements for all four treatments were finished
154 within 50 days. All t_3 measurements were subsequently measured after this period (see SI Fig.
155 S2 for exact specifications of the timing of t_1, t_2 and t_3 for all treatments). Each soil core was
156 followed until no more N_2O evolved (up till 7 days) before the next soil core was measured,
157 hence only one soil core for microsensor measurements was flooded at a time. Soil remained
158 sieved but unpacked at 4^{o}C and unamended with ^{15}N, with these procedures performed prior
159 to microsensor measurements starting.

160 ***2.2 Microsensors***

161 A standard N_2O microsensor (N_2O-100, Unisense, Science Park, DK-8000 Aarhus,
162 Denmark), a redox microsensor (RD-100, Unisense) and a redox reference electrode (REF-
163 RM, Unisense) were used to measure the N_2O concentration and the redox potential from the
164 soil:water interface, down through the soil profile at 500 µm steps (71 points per profile), with
165 movement controlled by a motorized micromanipulator. To ensure a complete mixing of the
166 water phase above the soil, an ambient air flow was generated over the water surface to avoid
167 N_2O accumulation in the water overlying the soil core. To ensure the soil water remained at a
168 constant level at all times, water was added several times daily to offset any evaporation,
169 maintaining a 1 cm water depth above the soil surface. The output current for the N_2O
170 microsensor was measured using a Microsensor Multimeter while the redox signal was
171 measured using a pH/mV-METER. The N_2O microsensor was calibrated with a five-point
172 calibration using a saturated N_2O solution to make standard solutions increasing by 100 µM
173 each step. Repeated calibration, after profiling, revealed that instrument drift was
174 insignificant. Each soil core profile was measured every two hours for 7 days or until the N_2O
175 concentration was below the detection limit (< 0.1 µM) at all depths. The room temperature
176 during the microsensor measurements was in the range of 21-23°C. Based on the microsensor

measurements, contour maps of the subsurface N_2O concentration and the redox potential were constructed using kriging interpolation (Surfer Version 9.785, Golden Software Inc., Colorado, USA).

2.2.1 Flux determination

The observed flux of N_2O from the flooded soil core was determined as the diffusive gas exchange across the diffusive boundary layer (DBL) according to Elberling and Damgaard (2001). The DBL is a thin film of water at the soil:water interface (often < 2 mm) where the only form of transport is molecular diffusion (Gundersen and Jørgensen, 1990). The linear concentration gradient over the DBL was used to determine the N_2O flux across the DBL using Fick's law (Clough et al., 2005). The diffusion coefficient for N_2O in water at 20°C was taken to be 2.2295×10^{-5} cm^2 s^{-1} (Ramsing and Gundersen, 2009). Verification of the flux was made based on dark, closed chambers (INNOVA 1313, LumaSense, Inc., Ballerup, Denmark), where the observed flux was compared to chamber measurements performed immediately after the microsensor measurements (see SI Fig. S3).

2.2.2 Modelling N_2O production and consumption

Assuming the N_2O concentration profile represents a pseudo-steady state, the SensorTrace PRO 3.0 programme (Unisense A/S, Science Park, DK-8000 Aarhus, Denmark) was used to model the depth-specific N_2O production and consumption zones. The programme is based on the model PROFIL by Berg et al. (1998). Here the effective gas diffusion coefficient through the soil is described as a function of the water-filled pore space, the soil porosity and the associated gas diffusion coefficient through air (Berg et al., 1998).

2.3 KCl extractions

Based on the preliminary results of the maximum N_2O concentrations in the soil core, measured using the microsensors, 8 time steps for the KCl extractions were chosen for each treatment distributed over time: One extraction was made at time zero (and subsequently used as a reference for the initial conditions), three were made between time zero and maximum N_2O concentration where a further extraction was made, and then two further extractions taken after the maximum N_2O concentration and the final extraction was taken when N_2O was no longer measurable (for details, see SI Fig. S1).

Prior to KCl extraction, the flooded cores were removed to drain for app. 10 minutes. A subsample of the soil, 10 g, from the top (0-1.2 cm) of the core was mixed with 60 mL 2 M KCl in a 107 mL glass bottle capped with an aluminium screw-top lined with a rubber septum leaving a headspace of 43 mL and vigorously shaken (30 seconds). Gas samples (8 mL) were then collected from the headspace, using a gas-tight syringe fitted with a stop-cock to prevent under-pressurisation of the sample, and stored in a pre-evacuated 6 mL Exetainer®. For all $^{15}N\text{-}NO_3^-$ treated samples an additional 16 mL gas sample was collected in a similar manner and placed in a pre-evacuated 12 mL Exetainer®. The soil/KCl solution was then shaken for 1 hour on an end-over-end shaker, left to settle for 5 minutes and filtered through Whatman 42 filter paper into a 30 mL sample vial and frozen (-20^{o}C) until analysis. The KCl extraction was repeated with a soil sample from the middle of the soil core (1.2-2.3 cm) and from the bottom (2.3-3.5 cm). The remainder of the soil at each depth was weighed and dried to determine the gravimetric water content.

Gas samples in the 6 mL Exetainers were analysed for methane (CH_4) and N_2O using a SRI 8610C gas chromatograph (SRI, Ca. USA) linked to a Gilson 222XL autosampler. CH_4 was determined using a flame ionisation detector (FID) while N_2O was determined with an electron capture detector (ECD) calibrated with certified gas standards (BOC gases) that

223 covered the range of sample concentrations (Mosier and Mack, 1980). The gas samples in the
224 12 mL Exetainers were analysed for ^{15}N-N_2O and ^{15}N-N_2 using a continuous flow isotope
225 ratio mass spectrometer (IRMS) on a Sercon 20-20 IRMS with ^{15}N calculations performed
226 according to the equations of Mulvaney and Boast (1986) and Stevens et al. (1993). When
227 calculating the release of ^{15}N labelled gas entrapped in the soil, corrections were made to
228 allow for headspace gas dissolved in the KCl solution using the appropriate Bunsen
229 coefficient (Moraghan and Buresh, 1977). Derived ^{15}N data permitted the transformation and
230 redistribution of the original $^{15}NO_3^-$ to be observed across inorganic-N and gaseous N species
231 at the times KCl extractions were performed.

232 Soil KCl extracts were thawed at room temperature and analysed for NH_4^+-N and NO_3^--N
233 concentrations using an Alpkem FS3000 twin channel flow injection analyser (FIA) with
234 Alpkem Winflow 4.03 software. Following the method of Brooks et al. (1989) sub-samples of
235 the KCl extracts were diffused and analysed to determine their NH_4^+-^{15}N and NO_3^--^{15}N
236 enrichments.

237 ***2.4 Statistical analyses***

238 Statistical analyses of Pearson product-moment correlation, F-test and one-way ANOVA
239 analyses were performed using the SPSS statistical package (v. 19.0), with a significance
240 level of at least 95 % (*$P < 0.05$). For KCl extractions three replicates were analysed ($n = 3$)
241 while for microsensor N_2O determinations two replicates were analysed ($n= 2$), t_1 and t_2.

3. Results

The initial values of the soil pH, NO_3^- concentration and DOC concentration as well as the effect of liming on the parameters can be seen in Table 1.

3.1 N_2O dynamics

For all microsensor measurements the repeated measurements at t_1 and t_2 can be seen as replicates because of the low variation (see SI Fig. S4) whereas t_3 differs markedly. For all treatments, the maximum N_2O concentrations were lower and peaked in half the time compared with t_1 and t_2, and at much lower levels. In the following, results will be based on t_1 and t_2, (see SI Fig. S4 for comments on t_3).

Regardless of treatment the N_2O concentrations initially increased and then decreased over time but with differences in the timing of the maximum concentration (26-34 hrs) and the maximum magnitude of the N_2O concentration (Fig. 1 and SI Fig. S5). There was a significant correlation between the observed N_2O diffusion flux and the maximum N_2O concentration in the soil ($r = 0.94$, $n = 256$, ***$P < 0.0001$) (Fig. 1a). Both parameters had a skewed bell-shaped pattern with a steep increase followed by a steep decrease after which they levelled out app. 72 hrs after flooding. The highest N_2O concentrations occurred in treatment TN, followed by TL, TC and TLN. The maximum N_2O diffusion flux was $2.5 \cdot 10^{-11}$, $3.8 \cdot 10^{-11}$, $5.7 \cdot 10^{-11}$ and $2.9 \cdot 10^{-11}$ mol N_2O cm^{-2} s^{-1} for treatments TC, TL, TN and TLN, respectively. N_2O fluxes obtained using microsensor measurements are in agreement with levels obtained by INNOVA chambers (SI Fig. S3). During the first 96 hrs of flooding, the mean (± stdev, $n = 2$) integrated N_2O fluxes for treatments TC, TL, TN and TLN were 29 ± 4.3, 41 ± 0.9, 54 ± 5.2 and 39 ± 8.9 mmol N_2O m^{-2} respectively.

After the initial short lag phase of app. 5 hrs the N_2O concentrations increased primarily in the zone 1-2 cm below the soil surface (Fig. 1b). Concentrations of N_2O below 2 cm depth decreased faster than in shallower depths and reached zero within 24-50 hrs after flooding (Fig. 1b and SI Fig. S5). After the maximum N_2O concentrations in the soil profile occurred, they then decreased with time in all depths still with the highest concentrations in the middle zone of the soil profile, until the N_2O concentrations were zero at all depths.

The decrease in NO_3^- concentrations correlated negatively with increasing N_2O concentrations (Fig. 1d) within the first 24-36 hrs (three time steps) for all four treatments. Subsequently, the rate of NO_3^- decrease reached a minimum over the last three time steps. For treatment TLN, the rate difference in NO_3^- decrease was small between the first and the last three time steps and the NO_3^- concentration did not reach zero during the experiment. Despite this, the N_2O concentrations were below detection for treatment TLN after 96 hrs.

As a consequence of flooding, the soil redox potential decreased over time with the rate of decrease increasing with increasing soil depth (Fig. 1c and SI Fig. S5). This depth driven change in redox potential happened primarily after the first 16 hrs of flooding. The primary redox range, where N_2O accumulates in the soil, for each of the treatments can be seen in Table 1. High N_2O concentrations were found in the same range for treatments TC, TN and TLN while the redox range for treatment TL was 100 mV lower. The N_2O concentrations and the redox potentials were significantly ($^*P < 0.05$) correlated (Table 1), as the N_2O concentrations portray a bell-shaped curve over time with a decreasing redox potential where a ‘build-up’ and a ‘build-down’ phase are divided around the occurrence of the maximum N_2O concentration (see SI Fig. S6).

3.2 N_2O production and consumption

The depth-specific consumption and production rates over time were modelled using the SensorTrace PRO 3.0 programme for each treatment, while assuming that the porosity and the effective diffusion coefficient were uniform throughout the packed soil core. The diffusion flux was simultaneously determined for each profile. In Fig. 2, 6 time intervals of the depth-specific activity are shown for treatments TC and TN. The corresponding figure for TL and TLN can be seen in SI (Fig. S7). The production (positive activity rates) of N_2O commenced after 10 hrs, primarily right below the DBL. The activity was several orders of magnitude larger for treatment TN compared to TC. After 20 hrs of flooding, N_2O was produced in the near-surface zone (2 cm) of the soil while consumption of N_2O (negative activity rates) increased below this depth. Thirty hrs after flooding, high production and maximum consumption rates were found, with consumption in the top (< 0.5 cm) and below 1.5 cm and production in a 1 cm zone from 0.5 to 1.5 cm below the surface. This pattern remained throughout the experiment, with rates of production and consumption decreasing with time. The model was successfully validated as a significant correlation, with a slope of 0.9, was found between the observed flux and the modelled flux ($r = 0.77$, $n = 754$, ***$P < 0.0001$).

Based on the time-integrated modelled diffusion flux and the time and depth-integrated N_2O production, more than one-third of N_2O produced in the soil was consumed within the soil and not released. Consumption accounted for 41 ± 6.9, 34 ± 3.7, 51 ± 0.4 and 48 ± 10.9% of N_2O produced for treatments TC, TL, TN and TLN, respectively.

3.3 ^{15}N recovery

Percentage recovery of ^{15}N as NO_3^-, N_2O, N_2 and NH_4^+ over time for treatments TN and TLN can be seen in Fig. 3. The atom% ^{15}N enrichment for N_2O, NO_3^- and NH_4^+ as well as $^{15}X_N$ for N_2 (the mole fraction of ^{15}N in the N pool from which the N_2 was derived) are shown in SI

(Fig. S8). At time 0 all added ^{15}N was present as NO_3^-. Over time recovery of ^{15}N in the NO_3^--^{15}N labelled pool is reduced while the other components increase. ^{15}N recovered as N_2O increased for the first 6 time steps (38 and 48 hrs after flooding for treatments TN and TLN respectively) after which it decreased to zero at 96 and 144 hrs after flooding. ^{15}N recovered as NH_4^+ increased steadily over the entire flooding period and ended up constituting 1.0 and 0.6% of the ^{15}N initially added for treatments TN and TLN, respectively. ^{15}N recovered as N_2 also increased over time. For treatment TN the increase was slow for the first 38 hrs, subsequently it increased rapidly to 115 ± 12% ^{15}N recovered at 72 hrs and levels out at 108 ± 13% after 96 hrs within the same range of the standard deviation. For treatment TLN the ^{15}N recovery of N_2 was close to zero within the first 72 hrs. After 144 hrs of flooding ^{15}N-N_2 constituted 26% of the initially added ^{15}N label. The recovered $^{15}N_2$ is displayed without standard deviations as less replicates are available as some fluxes were too low to be detected with only one replicate available in some cases. This is primarily the case for treatment TLN, and results should only be seen as best estimates.

4. Discussion

The soil profile N_2O concentrations measured using the microsensor are in the same range as the methodologically comparable study by Jørgensen and Elberling (2012), as the maximum N_2O concentration for TC is a factor 1.5 higher and the maximum N_2O diffusion flux is a factor of 4 higher than their measurements on a flooded temperate peat soil. However, the duration of the high N_2O emissions is longer when compared to other studies, resulting in an accumulated N_2O release (29-54 mmol N_2O m^{-2} for the four treatments) that is at least a factor of 10 higher: For the study by Jørgensen and Elberling (2012), the duration was 40 hrs, resulting in an accumulated N_2O release of 0.06 mmol N_2O m^{-2}, while an experimentally flooded natural tropical wetland soil resulted in peak emissions of 2.92 and 3.7 mmol N_2O m^{-2} for a 2.3 and 3 day peak duration (Liengaard et al., 2013).

There is a lack of studies examining peak N_2O emissions during flooding events from temperate agricultural fields. Choudhary et al. (2001) found the annual N_2O emission for a conventionally grown maize field in New Zealand to be 8.5-12.2 mmol N_2O m^{-2} yr^{-1} while Roelandt et al. (2005), summarizing the data from 30 studies recording the annual N_2O emissions from croplands and grasslands in North America and Europe, found emissions varied from 0.7 to 20.7 mmol N_2O m^{-2} yr^{-1}. These reported values are of the same order or smaller than the peak emissions found in this study. Thus, the non-steady state emissions, over just a single four day flooding period, reported here, can potentially contribute more N_2O to the atmosphere than the annual emissions of N_2O on croplands and grasslands. This emphasizes the importance of incorporating flooding events in studies of annual N_2O emissions and the need for further in-situ measurement of N_2O fluxes during flood events.

4.1 Treatment effects

347 During flooding redox conditions and N_2O concentrations were markedly affected. The pH of
348 the soil is also likely to have increased in all treatments following flooding due to reduction
349 processes such as Fe and Mn oxide reduction (Yu and Patrick, 2003) and denitrification
350 (Zárate-Valdez et al., 2006). Thus pH effects on N_2O emission are therefore a consequence of
351 both the direct effect of liming and the indirect effects of redox processes.

352 The redox potential in the soil was a time- and depth-specific parameter during flooding
353 (Fig. 1c). The fact that the reduction was faster in the bottom part of the soil relative to the top
354 is in line with the fact that reduction in the top will be counter-balanced by diffusion of
355 atmospheric O_2 into the soil core. A lower redox potential at a higher pH is in agreement with
356 the negative correlation between the two parameters described by Yu & Patrick (2003). The
357 range in values of these parameters is likewise in agreement with their findings.

358 During flooding, the development of the subsurface N_2O concentration over time portrays
359 the same bell-shaped profile and the same depth-specific distribution, independent of
360 treatment, also described by Liengaard et al. (2013). Higher NO_3^- concentrations resulted in
361 higher N_2O concentrations and emissions, except in treatment TLN. The reduced N_2O
362 production due to higher soil pH was significant when comparing TN and TLN, as expected
363 (Šimek & Cooper 2002), whereas this effect was not seen between TC and TL.

364 The unambiguous depth-specific distribution of the N_2O concentration can be explained by
365 the correlation between N_2O concentration and redox potential based on the distinct redox
366 ranges for N_2O accumulation and reduction. The low N_2O concentration in the top soil can be
367 explained by the combination of redox potentials being too high for N_2O accumulation and by
368 the diffusion of N_2O to the atmosphere. The level of reduction in the middle part of the soil
369 displayed the optimum redox potential where the denitrification process is promoted, but not

370 to an extent where N_2O was rapidly reduced to N_2. The low N_2O concentrations found in the
371 bottom part of the soil was either due to the fact that the low redox potentials favoured
372 complete denitrification, not allowing N_2O to accumulate, or that the denitrification process
373 was completely inhibited, allowing NO_3^- to be preserved (as seen for treatment TLN), where
374 any measured N_2O was a result of diffusion of N_2O produced in the middle zone. Subsurface
375 accumulation of N_2O and resulting emissions are therefore time-dependent as the redox
376 potential of flooded soils will continuously decrease.

377 The depth-specific distribution of the N_2O concentrations and the finding that distinct zones
378 of production and consumption of N_2O occur underlines the fact that spatial and temporal
379 changes in denitrification rates are not a sequential process, but rather a consequence of micro
380 zones of specific environmental conditions affecting the N_2O dynamics. More than one-third
381 of the produced N_2O in the soil was consumed within the soil, with the highest ratios for
382 treatments TN and TLN with high NO_3^- concentrations. The high N_2O production rate
383 observed for treatment TN was counterbalanced by high consumption rates. The consumption
384 fraction of N_2O is low when compared to the study by Liengaard et al. (2013) where about
385 two-thirds of the produced N_2O was consumed within the soil. The balance between
386 produced, consumed and emitted N_2O is therefore not a universal value, but a soil and
387 environment dependent value.

388 The net effect of flooding, liming and addition of N was reduced N_2O emissions, as
389 treatment TLN had the lowest emission during the time of study. The high NO_3^- concentration
390 of treatment TLN and the lower maximum N_2O concentration indicate that the denitrification
391 process was limited even at the NO_3^- reducing step. The lack of NO_3^- reduction explains the
392 higher redox potential, as the soil was not exhausted of easily available electron acceptors.
393 The limitation was not caused by the addition of ^{15}N as the incomplete reduction of $^{15}N\text{-}NO_3^-$

was of the same magnitude as the total NO_3^- reduction over time, dismissing isotope fractionation as the cause. Additionally, Pan et al. (2012) did not find NO_3^- reduction to be sensitive to pH variations within the pH range of the present study. Contrary to the other treatments, no CH_4 production was seen for TLN (see SI Fig. S9) despite the fact that a higher pH should increase the production (Le Mer and Roger, 2001). Thus, the net effect of the treatment on the gas producing processes remains uncertain.

Small concentrations of CH_4 were produced during flooding (see SI Fig. S9). Compared to N_2O, CH_4 is not a significant greenhouse gas to consider during short term floodings as the redox potential in the soil is not fully reduced to levels (< -100 mV) where CH_4 is the primary end product of mineralisation over CO_2 (Yu and Patrick, 2003).

4.2 Pathways of N transformation

The primary production mechanism of N_2O in the anoxic soil environment is denitrification. The intermediate products of denitrification were detected following the reduction of ^{15}N labelled NO_3^- (Fig. 3). The ^{15}N not accounted for 14-26 hrs after flooding is expected to be found as NO_2^- or NO. The recovery of ^{15}N was quantitative in treatment TN with effectively 100% of ^{15}N recovered as N_2 after 72 hrs. However, this was not the case for treatment TLN where after 144 hrs only 26% of the ^{15}N was recovered as N_2 with total ^{15}N recovery of 54 ± 5%. Reasons for the lower recovery in TLN may be due to volatilization of NH_4^+ to NH_3 enhanced by the higher pH (Sommer and Hutchings, 2001), as the NH_4^+ concentration was lower in treatment TLN compared to TN (see SI Fig. S9) or that the residual ^{15}N was incorporated in other non-measured N pools. Alternatively, a major difference between the TN and TLN treatments at the end of the 144 hours was the higher NO_3^- concentration in the TLN treatment. Thus if any NO_3^- was lost as the soil cores were drained prior to performing

417 the KCl extracts then this may have had a greater impact on the ^{15}N balance in the TLN
418 treatment. Another pathway may contribute to N_2O production. Only a small fraction of the
419 applied ^{15}N was recovered as NH_4^+ for both treatments (Fig. 3). Although small, the increase
420 over time indicates that NH_4^+ has been produced from DNRA of the applied $^{15}N\text{-}NO_3^-$ (Buresh
421 and Patrick, 1978). The NH_4^+ produced from the applied $^{15}N\text{-}NO_3^-$ was minimal when
422 compared to the total NH_4^+ concentration during the flooding event (see SI Fig. S9), but the
423 process could potentially be important if flooding continued for prolonged periods. It also
424 cannot be dismissed that the increase in $^{15}N\text{-}NH_4^+$ was caused by a release of assimilatory
425 reduced $^{15}N\text{-}NO_3^-$ during extraction, even though the extraction setup should not destroy
426 microbial cells.

427 ***4.3 N_2O emissions and agricultural management practice under future climate conditions***

428 Based on these results, a marked reduction in N_2O production and emission during soil
429 flooding could be achieved if soils were limed prior to tillage. As the reduction is achieved
430 with only an increase in pH of 1.3 units it emphasises the importance of liming often, to keep
431 the pH constant. Low lying areas are of highest risk of flooding and are also likely to receive
432 additional inputs of N from the surrounding elevated areas. To reduce the risk of flood-
433 induced N_2O emissions, N-application in low lying areas should be minimized and where
434 possible these areas should be drained. If flooded, drainage should be avoided, as it is at the
435 boundary between aerobic and anaerobic conditions when N_2O accumulation is seen.
436 Consequently, there is a need for additional experiments to include more soil types and land
437 uses before implications are scaled to larger areas.

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Supporting information

544 **Table 1.** Overview of soil pH, NO_3^- and DOC concentration as well as the absolute and the
545 primary redox range and the redox range for the 'build-up' and 'build-down' phase of N_2O
546 for all four treatments (n =3).

	Control (TC)	**Limed (TL)**	**N added (TN)**	**N added & limed (TLN)**
pH	6.1 ± 0.1	7.4 ± 0.1		
NO_3^- (µgN g soil^{-1})	97.8 ± 27.6	108.9 ± 49.9	197.9 ± 73.3	183.3 ± 31.8
DOC (µg g soil^{-1})	129.4 ± 3.9	160.9 ± 2.7		
Primary redox range (mV)	300-450	200-350	250-450	300-400
N_2O build-up redox range (mV)	414-339	335-242	418-312	384-331
N_2O build-down redox range (mV)	366-289	298-194	342-232	361-282

547

548 **Figure 1.** Development of mean maximum N_2O concentration and observed diffusion flux
549 (a), time- and depth-specific contour plot of the N_2O concentration over time for treatment TC
550 (b), time- and depth-specific contour plot of the redox potential over time for treatment TC (c)
551 and the sum of the NO_3^- concentration in the soil core over time (d). For contour plots of
552 treatment TL, TN and TLN see SI (Fig. S5; 5A, 5B and 5C respectively).

553

554 **Figure 2.** Modelled activity of N_2O production (positive values) and consumption (negative
555 values) over 6 time intervals (10, 20, 30, 50, 70 and 90 hrs after flooding) for treatment TC
556 (solid line) and TN (dashed line). Values < 0.0007 mol cm^{-3} s^{-1} are not shown.

557

558 **Figure 3.** ^{15}N recovered as NO_3^-, N_2O, N_2 and NH_4^+ (secondary axis) over time for treatment
559 TN (a) and TLN (b). All values are normalized against the recovered ^{15}N-NO_3^- at time 0 (n =
560 3).